Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association 81 (2018) 262–267

www.jcma-online.com

Original Article

Antiviral effects of *Lactobacillus crispatus* against HSV-2 in mammalian cell lines

Elham Mousavi [a,c], Manoochehr Makvandi [a,c], Ali Teimoori [a,c], Angila Ataei [b], Shokouh Ghafari [a,c], Alireza Samarbaf-Zadeh [a,c,*]

[a] *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[b] *Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[c] *Department of Virology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Received May 26, 2017; accepted July 24, 2017

Abstract

Background: Herpes simplex virus type 2 (HSV-2) infectious disease is one of the most common viral sexually transmitted diseases. As regards, vaginal *lactobacilli* play an important role in protecting host against the urogenital pathogens; here we assessed the potential antiviral activity of *Lactobacillus crispatus* against HSV-2 infection in vitro.
Methods: Both Vero and HeLa cell lines were treated by *L. crispatus* before, during and after HSV-2 infection. The pre-incubation assay was also performed for the evaluating of virus adsorption by *L. crispatus*. Virus titer reduction in each stage was determined by a plaque reduction assay.
Results: *L. crispatus* significantly decreased the infectivity of the HSV-2 in initial steps on both cell lines; however, no significant inhibition was ascertained during adsorption and multiplication process. The *lactobacilli* adhere on Vero cells two-fold stronger than HeLa and subsequently protect the Vero cells nearly 2.5 fold higher than HeLa cell against the virion. Co-incubation of HSV-2 with bacterial cells prior to virus inoculation significantly decreased the virus titer.
Conclusion: *L. crispatus* appears to inhibit the entry of the virus into cells by trapping HSV-2 particles. In addition, formation of *L. crispatus* microcolonies in the cell surface could block HSV-2 receptors and prevent viral entry to cells in initial infection steps.
Copyright © 2017, the Chinese Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: Antiviral activity; *HeLa* cell line; HSV-2; *Lactobacillus crispatus*

1. Introduction

Herpes simplex virus (HSV-2) is a member of the herpesviridiae family and the principal species responsible for genital herpes.[1] HSV-2 produces localized lesions on skin and mucus membranes of infected individuals, predisposing partners to acquired HIV during sexual activity.[2] Several drugs, such as acyclovir, valacyclovir, and famciclovir are used to treat HSV-2. Resistance to the many of these drugs, especially acyclovir, has been increasingly reported.[3] Therefore, new strategies for prophylactic and treatment of HSV-2 infection are necessary.

It has been reported that any disturbance in the population of normal microbiota of the vagina gives rise to a risk of urogenital tract infections, such as bacterial vaginosis, candidiasis, and viral sexually transmitted diseases.[4,5] Clinical studies have demonstrated that an abnormal vaginal flora is a risk factor for harboring HSV-2 and human papillomavirus (HPV) viruses.[6,7] *Lactobacilli* are part of the vaginal microbiota of healthy females,[8] and several studies have investigated

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Alireza Samarbaf-Zadeh, Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Golestan Ave., University Campus, P.O. Box 169, Ahvaz, Iran.
E-mail address: alirezasamarbaf_78@hotmail.com (A. Samarbaf-Zadeh).

http://dx.doi.org/10.1016/j.jcma.2017.07.010
1726-4901/Copyright © 2017, the Chinese Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

the inhibitory effect of different lactobacillus species against HSV, but the mechanism of action of *lactobacilli* in combating HSV infection remains unclear.[9–12] Numerous mechanisms have been proposed for the protection of the epithelial layer of the vagina against invasion pathogens. These include secreted bacterial metabolites (lactic acid, hydrogen peroxide, and bacteriocin), stimulation of innate immunity, and competition for epithelial cell receptors.[13,14] One of the most important characteristics of *lactobacilli* is their ability to colonize epithelial and mucosal cells, thereby preventing invasion by pathogens of these cells.[15,16] *Lactobacilli* might inhibit viral multiplication via competition for attachment to cell receptors or via induction of epithelial cells to produce antimicrobial substances.[17] Normal vaginal microbiota has been composed with more than 20 species of *lactobacilli* that their characteristics and proportions vary in different ethnic groups.[18] As regards, *Lactobacillus crispatus* is one of the dominant species colonized in the healthy female vagina; it would be interesting to verify inhibitory effect of this strain against HSV-2.[19]

The Vero cell line, derived from African green monkey kidney cells, is competent for the replication of many human viruses, including HSV-2.[20] In the present study, to understand the probable interference of *lactobacilli* with the viral infection in the human cells, HeLa cells, derived from human tissues was used, as well as Vero cells, to examine the potential role of *L. crispatus* in combatting HSV-2 infection.

2. Methods

2.1. Virus, cell lines and bacteria

HSV-2 strain was purchased from Tarbiat Modares University, Tehran, Iran. The virus was propagated in Vero and HeLa cell lines (provided from Razi institute, Iran). A titer of 10^7 PFU/ml of the virus was determined by a plaque assay, and viral particles with a multiplicity of infection (MOI) of 0.01 were used in all the experiments. Both Vero and HeLa cells were maintained in Dulbecco's Modified Eagles Medium (DMEM, Gibco-Germany), supplemented with 10% fetal bovine serum (Gibco-Germany) and 1% penicillin/streptomycin (Gibco-Germany) and incubated at 37° C with 5% CO_2. Our previously reported isolate, *L. crispatus* KP090111, from the vagina of a healthy Iranian female was used as a probiotic candidate in this study.[21] The isolated bacterium was maintained in de Man Rogosa-Sharp (MRS, Merck-Germany) broth containing 20% glycerol at −70 °C. Viable microorganisms were counted by plating serial 10-fold dilutions of the bacterial suspension on MRS agar after incubation for 48 h. Based on colony-forming units, the number of viable bacteria in 1 ml of the suspension after 24 h was determined as 10^8 CFU/ml. Using spectrophotometry, the optical density at this concentration was equal to 2.

2.2. Determination of the cytotoxicity of L. crispatus

To evaluate the possible cytotoxicity of the *lactobacilli* in the cell lines, a serial dilution of bacteria was prepared, and each dilution was added to a cell monolayer of both Vero and HeLa cells cultured on 96-well plates. The survival ability of the cells was determined by methylthiazolyl-diphenyl-tetrazolium bromide (MTT) assay. Briefly, bacterial cells from an overnight suspension culture of *L. crispatus* were collected and washed with Phosphate-buffered saline (PBS). The pellet was resuspended in DMEM without antibiotics at different concentrations (10^4, 10^5, 10^6, 10^7, and 10^8 CFU/ml). Then, 100 µl of each bacterial dilution was added to each well and the microplate was incubated at 37° C with 5% CO_2 for 24 h. Cells were then washed with PBS three times, and 180 µl of fresh DMEM and 20 µl of MTT (5 mg/ml) were added to each well and the plate was incubated at 37° C for 4 h. Finally, after addition of DMSO, an ELISA reader (Tekan-Germany) read the optical density (OD) at 570 nm. The percentage of cell viability was calculated according to the Kassaa et al.[12] Cells with DMEM were used as a negative control.

2.3. Adhesion of L. crispatus to Vero and HeLa cells

The ability of bacterium to attach to the target cells was determined as previously described.[21] Briefly, overnight culture of *L. crispatus* was collected, washed with PBS, and resuspended in DMEM to a final concentration of 10^8 CFU/ml. Then a bacterial suspension with CFU/cell ratio of 100 was added to the monolayer of target cells in 24 well-plates. After 4 h incubation at 37 °C, the cell monolayer was washed with PBS three times to remove free bacteria from the well. The cells were detached from the well by trypsin 0.05% and resuspended in DMEM. The total number of viable bacteria adhered to the cells was determined by the colony counting method.

2.4. Antiviral activity of the bacterial supernatant

Overnight culture of *L. crispatus* was washed in PBS and 5×10^6 CFU resuspended in DMEM. The suspension was maintained at 37° C for 24–48 h. The bacterial suspension was then centrifuged for 10 min at 10,000 g at 4° C, the supernatant was collected, and the pH was neutralized with NaOH 1M in a sterile condition.

2.5. In vitro interference experiments

For interference studies, infection of the Vero and *HeLa* cells (concentration of 10^5 cells/well) with HSV-2 was performed at a MOI of 0.01. The suspension of *L. crispatus* was applied to the cell culture, at a concentration of 10^7 CFU/ml (100 CFU/cell), in three different stage of the HSV-2 life cycle that let us to know at which step during viral cycle, the addition of the lactobacillus is most effective. In the first series of the experiment, named *pre-treatment*, the cells were treated first with a suspension of *L. crispatus* for 1.5 h, and then supplemented with HSV-2 for 1 h. In second series of the experiments, named *competition*, the cells were concurrently exposed to the bacterium and HSV-2 for 1 h; they were also first exposed to the virus for 1 h and then to the bacteria without washing in the third series of experiments so called

post-infection. The schematic representation of our experimental setup for studying the interference was depicted in Supplementary Fig. 1. After 24 h incubation of the cells at 37° C, the supernatant of each well was collected and titered for infectivity of HSV-2 on Vero and HeLa cells by plaque reduction assay.

In another experiment, named *pre-incubation* (See S Fig. 1), direct interaction between bacteria and the virus particles in a cell free system was evaluated. Briefly, a microtube containing the *lactobacilli* and viral particles was incubated for 2 h at room temperature. The microtube was then centrifuged for 10 min at 4000 g and the supernatant was subjected to the aforementioned plaque assay analysis. All experiments were performed in triplicate for a minimum of six wells.

2.6. Plaque reduction assay

The titer of the virus was determined by plaque assay. Nearly, 4×10^5 cells/well were seeded in six well plates and incubated for 24−48 h to reach at least 98% of confluence. The collected supernatants were serially diluted and 500 µl of each dilution were added to each well. The plate was incubated at 37° C for 1 h to allow attachment of the virus to the cells. The supernatant was then removed from each well, and 3 ml of 1.5% DMEM/agar was added to each well. The plates were incubated for 48−72 h until the formation of plaques. Neutral red at a concentration of 0.01 was added to each well and incubated for 24 h. The plaques were then counted. A reduction in the number of plaques in each experiment was determined by comparison with a positive control (a suspension of the viruses with MOI of 0.01).

2.7. Statistical analysis

All calculations were performed with IBM SPSS software, version 22. ANOVA, followed by a Least Significant Difference (LSD) test that performed to compare the reduction in the viral titer in each of the experiments. The P value of <0.05 was considered statistically significant. All the data are given as the mean ± SD.

3. Results

3.1. Evaluation of the L. crispatus effect on the cell viability and its capability to attachment to the cells

Based on the cytotoxicity assay of *L. crispatus* on Vero and HeLa cells, the cell viability was dramatically decreased when the concentration of *lactobacilli* raised from 10^7 CFU/ml to 10^8 CFU/ml (Fig. 1). Thereby, an optimum concentration of 10^7 CFU/ml was applied in all the interference experiments.

The bacterial adhesion was measured after 2 h co-incubation of the bacteria and both Vero and HeLa cells. As shown in Fig. 2 the results were expressed as a percentage of the initial bacterial count.[22] The percentage of adhering bacteria to Vero and HeLa cells was 12% (SD = 2.5) and 5.8% (SD = 1.2) respectively.

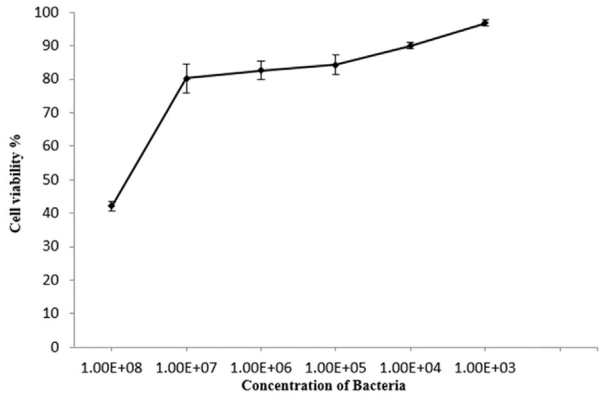

Fig. 1. Effect of *L. crispatus* on the viability of the cell line. Cell viability was tested by an MTT assay after 24 h. The concentration of bacteria with the least cytotoxic effect was 10^7 CFU/ml.

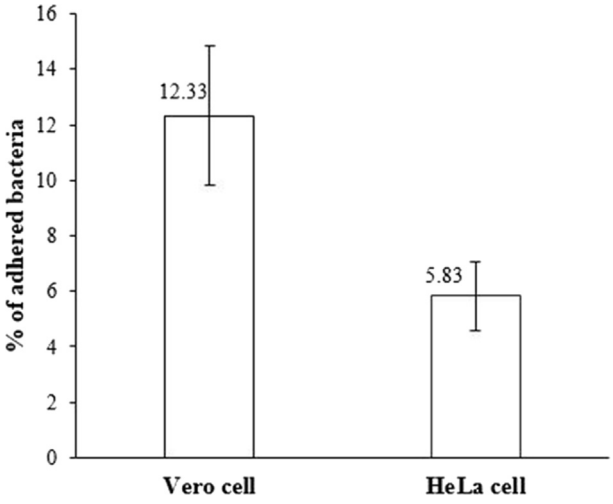

Fig. 2. The ability of *L. crispatus* to adhere to Vero and HeLa cells. Data was shown as % adhesion. The efficiency of adhesion on cell lines was 12.33% and 5.8% respectively.

3.2. Evaluation of virus titer reduction in the cells treated with L. crispatus

As delineated in Fig. 3, amongst the three interference experiments, a significant antiviral activity of the *L. crispatus* was shown in the *pre-treatment* step on both Hela and Vero cells. But, the titer of HSV-2 was more decreased in the Vero than HeLa cell line. However, in the both cell lines, neither in *competition* stage, when virus and bacteria simultaneously were presented on cells, nor in *post-infection*, which bacteria were added after adsorption of the virus into the cells, there was no significant reduction in the HSV-2 titer.

3.3. Virus adsorption by the bacteria in pre-incubation assay

The analysis of HSV-2 adsorption by *L. crispatus* showed that the virus titer of the supernatant was dramatically decreased after 2 h incubation of the bacteria and the viruses in a cell free condition (Fig. 3).

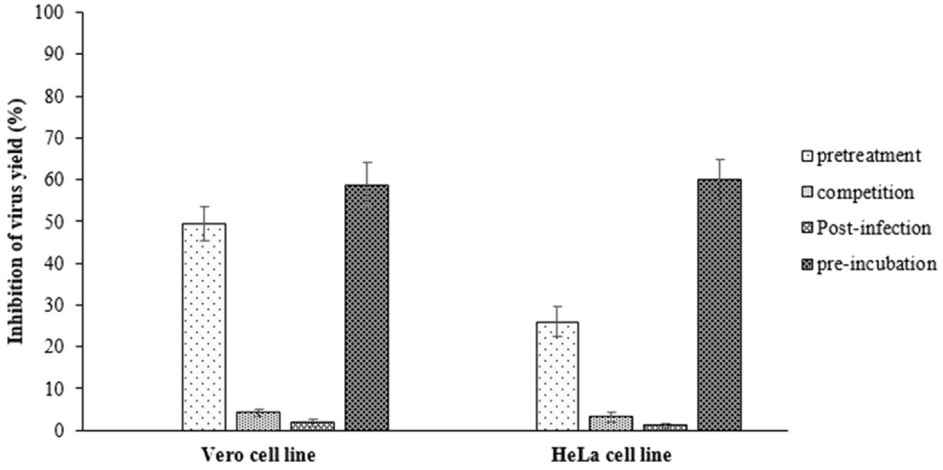

Fig. 3. Inhibition of HSV-2 multiplication by *L. crispatus*. The reduction of the virus titer was measured by a plaque assay. The results were mean ± standard deviations from three independent experiments.

3.4. Antiviral activity of the bacterial supernatant

No significant changes in the virus infectivity were observed after 2 h co-incubation of the bacterial supernatant and the HSV-2 at room temperature on both Vero and HeLa cell lines (Fig. 4).

4. Discussion

HSV-2 is one of the most prevalent etiologies of sexually transmitted diseases, a risk factor for acquiring HIV.[23] Clinical and animal studies have demonstrated that *lactobacilli* play an important role in the prevention of viral infections.[24] Vaginal microbiota inhibits the viral infection by several postulated mechanisms such as production of active metabolites, formation physical barrier against pathogen adhesion on epithelial cells,[24] physical interaction to the virus envelope[12,25] and stimulation of the immune system.[26] In this study, we assessed the potential anti-HSV activity of *L. crispatus*, which is one of the most dominant lactobacillus species isolated from healthy women vagina.[21,27]

It has been shown that *pre-treatment* of *Lactobacillus gasseri* strain on Vero cells dramatically inhibited the infectivity of HSV 1 & 2.[11,12] Consistently, on HeLa and Vero cell lines, *L. crispatus* significantly decreased the infectivity of the virion in initial steps by 26–49%, respectively (Fig. 3). However, presence of the metabolically active bacterial cells during or after infection process have the same results as control (Fig. 3). As previously reported *co-incubation* of the bacteria and HSV-2 at the CFU/PFU ratio of 1–100,000 on

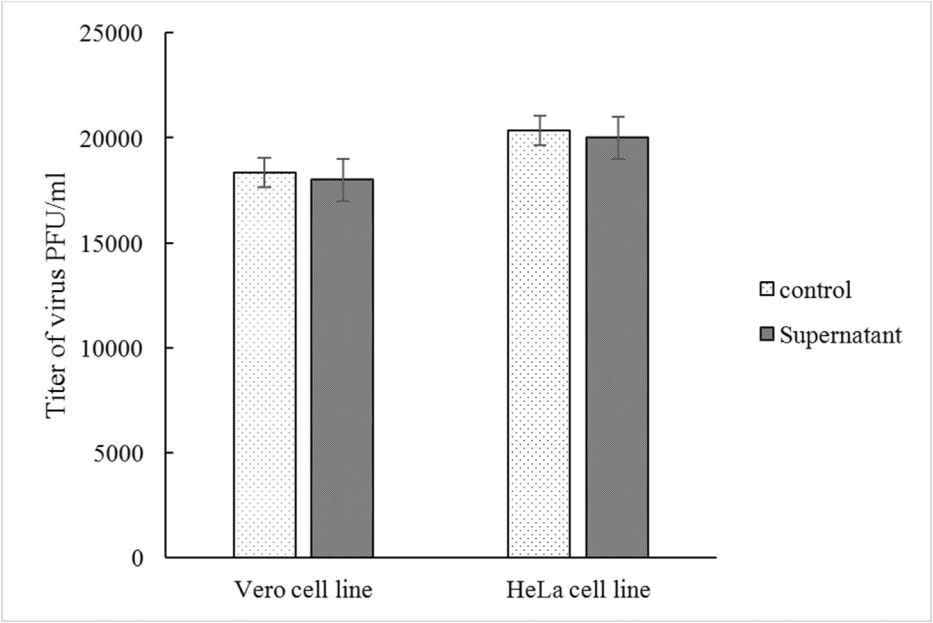

Fig. 4. Reduction of viral titer in bacterial supernatant. The results were mean ± standard deviations from three independent experiments.

cells resulted varied range of inhibition from 0 to 90%.[10,12] Similarly, our results showed that virus particles adsorbed more effectively than bacteria on cell membrane but antiviral activity increased when the PFU/CFU ratio reduced from 10 to 1 (data not shown). Also after entry of the virus particles to the cells no further inhibition was induced by the *lactobacilli* on intracellular virus replication process (Fig. 3). Overall, these results suggested that physical interaction or active metabolites of the *lactobacilli* inhibit/block the adsorption of HSV-2 to the cell membrane.[10,24,28] Some studies have shown that the excretion of metabolites, such as lactic acid, bacteriocin and H_2O_2, by *lactobacilli* was contributed in the inactivation of HSV particles before infection.[10,11] So, we examined the antiviral activity of the *L. crispatus* secreted metabolites as described by previous studies.[10,12] No significant antiviral activity was found in the supernatant when the bacteria were grown in DMEM media (Fig. 4). On the contrary, *co-incubation* of HSV-2 with bacterial cells prior to virus inoculation significantly decreased the virus titer by up to 60% (Fig. 3). In this concept, Lai et al. demonstrated that vaginal *lactobacilli* attachment to the surface of HIV prevented the diffusion stage of this virus.[29] Ivec et al. reported that *lactobacilli* were able to block virus entry into cells by trapping the VSV particles.[25] Kassa et al. also obtained similar results for the inactivation of HSV-2 particles with attachment of *L. gasseri* to viral envelope.[12] These findings are in line with the possibility of virus inactivation with physical interaction between *lactobacilli* and viral envelope.[30]

Besides, as shown in Fig. 3 the inhibitory effect of the *L. crispatus* on the Vero cells was approximately 2.5 fold higher than HeLa in initial infection steps. This may be described by nearly two-fold stronger adherence of *L. crispatus* to Vero cell line (Fig. 2). The first step of virus entry to a host cell is virus attachment to the cell receptors, and therefore inhibition of this attachment could be prevent disease process.[33] *Lactobacilli* may block virus entry to the host cell by masking specific or non-specific receptors.[15,28] Therefore, the second possibility of virus inactivation by *L. crispatus* is thought to be exerted through a physical barrier that formed by the bacterial cells on cell membrane.[28,30] The capability of *lactobacilli* to attach to the cells depends on various factors like the cell wall components of bacteria, cell receptors and soluble proteins secreted by the host cells.[31] Moreover, previous studies indicated that *L. crispatus* strains encode adhesion components named, S-layer proteins that play important role on adherence of bacteria on Hela cells.[32] However, more studies should be conducted on normal vaginal cell lines to elucidate the adhesion ability of *lactobacilli* on epithelial cells and protection against viral infections.

In conclusion, two mechanisms may be involved in the antiviral effect of *lactobacilli* towards HSV-2 (1.) formation of *L. crispatus* microcolonies in the cell surface could block HSV-2 receptors and prevent viral entry to cells in initial infection steps and (2.) entrapment of viral particles by direct interaction of *L. crispatus* with HSV-2 envelope.

Since the present results indicate a physical contact between bacterial cells and viral envelope could inhibit HSV-2 infection, it would be interesting to examine the potential prophylactic properties of *L. crispatus* cell wall components against HSV-2 infection in vitro and in vivo.

Acknowledgments

This study was done as part of a doctoral thesis (registration number: 92197) undertaken at the Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran. The authors are thankful to the Director of the Infectious and Tropical Disease Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran for the financial support to carry out this project.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcma.2017.07.010.

References

1. Field HJ, Hodge RAV. Recent developments in anti-herpesvirus drugs. *Br Med Bull* 2013;**106**:213−49.
2. Celum CL. The interaction between herpes simplex virus and human immunodeficiency virus. *Herpes* 2004;**11**(suppl 1):36A−45A.
3. Bacon TH, Levin MJ, Leary JJ, Sarisky RT, Sutton D. Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. *Clin Microbiol Rev* 2003;**16**:114−28.
4. Sewankambo N, Gray RH, Wawer MJ, Paxton L, McNairn D, Wabwire-Mangen F, et al. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. *Lancet* 1997;**350**:546−50.
5. Cohen CR, Duerr A, Pruithithada N, Rugpao S, Garcia P, Nelson K, et al. Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. *AIDS* 1995;**9**:1093−8.
6. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis* 2003;**37**:319−25.
7. Watts DH, Fazzari M, Minkoff H, Hillier SL, Sha B, Glesby M, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. *J Infect Dis* 2005;**191**:1129−39.
8. Reid G. Probiotic agents to protect the urogenital tract against infection. *Am J Clin Nutr* 2001;**73**(Suppl 1):437S−43S.
9. Khani S, Motamedifar M, Golmoghaddam H, Hosseini HM, Hashemizadeh Z. In vitro study of the effect of a probiotic bacterium *Lactobacillus rhamnosus* against herpes simplex virus type 1. *Braz J Infect Dis* 2012;**16**:129−35.
10. Conti C, Malacrino C, Mastromarino P. Inhibition of herpes simplex virus type 2 by vaginal *lactobacilli*. *J Physiol Pharmacol* 2009;**60**(Suppl 6):19−26.
11. Zabihollahi R, Motevaseli E, Sadat SM, Azizi-Saraji AR, Asaadi-Dalaie S, Modarressi MH. Inhibition of HIV and HSV infection by vaginal *lactobacilli* in vitro and in vivo. *Daru* 2012;**20**:53.
12. Al Kassaa I, Hober D, Hamze M, Caloone D, Dewilde A, Chihib N, et al. Vaginal *Lactobacillus gasseri* CMUL57 can inhibit herpes simplex type 2 but not Coxsackievirus B4E2. *Arch Microbiol* 2015;**197**:657−64.
13. Marco ML, Pavan S, Kleerebezem M. Towards understanding molecular modes of probiotic action. *Curr Opin Biotechnol* 2006;**17**:204−10.
14. Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. *Curr Opin Biotechnol* 2005;**16**:204−11.

15. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. *Antonie Leeuwenhoek* 2002;**82**:279−89.
16. Jafarei P, Ebrahimi MT. Lactobacillus acidophilus cell structure and application. *Afr J Microbiol Res* 2011;**5**:4033−42.
17. Deplancke B, Gaskins HR. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am J Clin Nutr* 2001;**73**:1131S−41S.
18. Lamont RF, Sobel JD, Akins RA, Hassan SS, Chaiworapongsa T, Pedro J, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. *BJOG* 2011;**118**:533−49.
19. Sethi S, Singh G, Sharma M. *Lactobacilli* as probiotics against genital infections. *Indian J Med Res* 2009;**129**:628−30.
20. Ammerman NC, Beier-Sexton M, Azad AF. Growth and maintenance of Vero cell lines. *Curr Protoc Microbiol* 2008 Nov. http://dx.doi.org/10.1002/9780471729259.mca04es11. A − 4E.
21. Mousavi E, Makvandi M, Teimoori A, Ataei A, Ghafari S, Najafian M, et al. In vitro adherence of Lactobacillus strains isolated from the vaginas of healthy Iranian women. *J Chin Med Assoc* 2016;**79**:665−71.
22. Letourneau J, Levesque C, Berthiaume F, Jacques M, Mourez M. In vitro assay of bacterial adhesion onto mammalian epithelial cells. *J Vis Exp* 2011 May 16;(51). pii: 2783.
23. Caldeira TD, Gonçalves CV, Oliveira GR, Fonseca TV, Gonçalves R, Amaral CT, et al. Prevalence of herpes simplex virus type 2 and risk factors associated with this infection in women in southern Brazil. *Rev Inst Med Trop Sao Paulo* 2013;**55**:315−21.
24. Petrova MI, Lievens E, Malik S, Imholz N, Lebeer S. Lactobacillus species as biomarkers and agents that can promote various aspects of vaginal health. *Front Physiol* 2015;**6**:81.
25. Ivec M, Botić T, Koren S, Jakobsen M, Weingartl H, Cencic A. Interactions of macrophages with probiotic bacteria lead to increased antiviral response against vesicular stomatitis virus. *Antivir Res* 2007;**75**:266−74.
26. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat Rev Microbiol* 2010;**8**:171−84.
27. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig S, McCulle SL, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci* 2011;**108**(Suppl 1):4680−7.
28. Juntunen M, Kirjavainen PV, Ouwehand AC, Salminen SJ, Isolauri E. Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clin Diagn Lab Immunol* 2001;**8**:293−6.
29. Lai SK, Hida K, Shukair S, Wang Y, Figueiredo A, Cone R, et al. Human immunodeficiency virus type 1 is trapped by acidic but not by neutralized human cervicovaginal mucus. *J Virol* 2009;**83**:11196−200.
30. Al Kassaa I, Hober D, Hamze M, Chihib NE, Drider D. Antiviral potential of lactic acid bacteria and their bacteriocins. *Probiotics Antimicrob Proteins* 2014;**6**:177−85.
31. Pizarro-Cerdá J, Cossart P. Bacterial adhesion and entry into host cells. *Cell* 2006;**124**:715−27.
32. Chen X, Xu J, Shuai J, Chen J, Zhang Z, Fang W. The S-layer proteins of *Lactobacillus crispatus* strain ZJ001 is responsible for competitive exclusion against *Escherichia coli* O157:H7 and *Salmonella typhimurium*. *Int J Food Microbiol* 2007;**115**:307−12.
33. Dimitrov DS. Virus entry: molecular mechanisms and biomedical applications. *Nat Rev Microb* 2004;**2**:109.